

I. AMENDMENTS TO THE CLAIMS

This listing of the claims will replace all prior versions and listings. Claims 1-7 are currently pending in this application.

LISTING OF CLAIMS:

Claim 1 (previously presented): A method of assembling PCR fragments, comprising

- a) making a first PCR fragment with first and second primers, wherein the second primer comprises a modified nucleotide that can be removed by a DNA repair enzyme, resulting in a 3' overhang, and wherein the first PCR fragment comprises a first site specific recombinase site;
- b) treating the first PCR fragment with a DNA repair enzyme to generate a 3' overhang and immobilizing the first PCR fragment on a solid support or vice versa;
- c) making a second PCR fragment with third and fourth primers, wherein the third and fourth primers each comprises a modified nucleotide that can be removed by a DNA repair enzyme resulting in a 3' overhang;
- d) treating the second PCR fragment with a DNA repair enzyme to generate a 3' overhang;
- e) annealing and ligating the first and second PCR fragments;
- f) optionally repeating steps c, d and e until a last PCR fragment is added to the growing chain to produce an assembled fragment, wherein the last PCR fragment comprises a second site specific recombinase site; and
- g) simultaneously removing and circularizing the assembled fragment from the solid support with a site specific recombinase in a single step.

Claim 2 (original): The method of claim 1, where one of the PCR fragments comprises an origin of replication and a selectable marker.

Claim 3 (original): The method of claim 1, wherein the first PCR fragment or the last PCR fragment comprises an origin of replication and a selectable marker.

Claim 4 (original): The method of claim 1, wherein the site specific recombinase is CRE and the site specific recombinase site is lox.

Claim 5 (original): The method of claim 1, wherein the nucleotide is deoxyuridine and the DNA repair enzyme is uracil-DNA-glycosylase followed by T₄ endonuclease V.

Claim 6 (original): The method of claim 5, wherein the assembled DNA is greater than 30 kb.

Claim 7 (original): The method of claim 5, wherein the assembled DNA is greater than 30, 40, 50, 75, 100, 125, 150, 200, 250 , 300, 350, 400, 450, 500, 750, 1000 or 1500 kb.